CHANGES IN SCALP HAIR ROOTS AS A MEASURE OF TOXICITY FROM CANCER CHEMOTHERAPEUTIC DRUGS*

ROBERT G. CROUNSE, M.D.† AND EUGENE J. VAN SCOTT, M.D.

Most of the drugs used in cancer chemotherapy affect the growth and metabolism of not only malignant cells, but certain normal tissues as well. In general, those tissues with more rapid metabolic and mitotic rates such as the bone marrow, gastro-intestinal epithelium, and oral mucosa are most noticeably affected, and damage to these tissues provides the most common measurable signs of "toxicity" observed during the administration of these agents.

The roots of growing human scalp hairs also have a high metabolic and mitotic activity. The germinative tissue of an average hair root, for example, produces 0.35 mm. of hair shaft every twenty-four hours (1), and in so doing, each day approximately reduplicates its entire cell population (2). It is not surprising therefore, that these same anti-cancer agents may interfere markedly with normal function of the hair root.

Damage to hair roots is not generally recognized in patients unless the interference with growth is severe enough to cause alopecia. Acute hair loss can result only from a rather profound disturbance of the growing hair root, since such loss is the ultimate response in a series of graded anatomical or biochemical alterations, each proportionate to the degree of disturbance. Thus, loss of hair following administration of Aminopterin® has been reported to be correlated with drug dosage (3), and anatomical alterations in hair roots following Methotrexate® (10-methyl-aminopterin) have been observed in the absence of clinical alopecia (4).

Several points of interest arise from these considerations. What agents cause identifiable alterations in the hair root? Are the changes in each instance similar or are there significant differences? What are the conditions necessary for such damage? Of what practical value might such information be, either in the clinical management of the patient or in assessment of drug action?

The present study was undertaken to investigate these questions by means of microscopic examination of scalp hair roots epilated from patients receiving a variety of chemotherapeutic agents, and comparing them with hairs from patients receiving no drugs and from persons without known illness. Changes in hairs, when observed, were further compared with hematologic alterations and other clinical signs secondary to either drug or disease.

METHODS AND MATERIALS

For each examination, approximately one hundred scalp hairs were manually epilated by means of needle-holding forceps, one jaw of which was coated with solder to assure a uniformly tight closure of the jaws and prevent slipping of the hairs grasped therein. The root ends of the hairs were cut off with scissors, evenly distributed in water in a small Petri dish with a grid etched on the bottom surface, and examined under a low-power (dissecting) microscope.

Due to cyclically occurring periods of growth and quiescence of hair, any large sampling of hair from a given area may include both growing (anagen) (Fig. 1A, 1B) and quiescent (telogen) (Fig. 1C) types. In the human occipital scalp approximately 86% of the hairs have been found to be growing, the remainder quiescent (4). In the present work, hairs were classified according to the stage of the growth cycle in order to detect possible variations from the normal ratio of growing/resting, and in addition were examined for the presence and structural variation of their internal and external root sheaths, the appearance of the germinative matrix of the hair bulb, and the contour of the more distal keratogenous zone and keratinized shaft.

As normal controls hairs were examined from a group of 38 healthy persons which included physicians, nurses, laboratory technicians and

* From the Dermatology Service, General Medicine Branch, National Cancer Institute, (National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare) Bethesda, Maryland.
† Present address: Section of Dermatology, Department of Medicine, University of Miami School of Medicine, Jackson Memorial Hospital, Miami, Florida.
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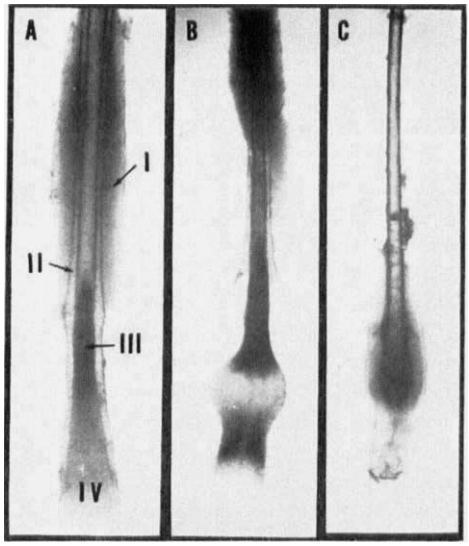

FIG. 1. Epilated normal scalp hair roots. A. Growing (anagen) root with external sheath (I) and internal sheath (II) intact. Keratogenous zone (III) appears dark in transmitted light. The mitotically active zone, the germinative matrix, is in the lowermost portion of the root (IV). B. Appearance of growing hair root in the absence of intact internal and external root sheaths. The absence of peribulbar sheaths permits the bulb to flare. C. Resting (telogen) hair root. Such roots, the bulbs of which are keratinized and mitotically inactive, are not affected by drugs.

other hospital employees whose ages ranged from 17–55 years. Hairs from the occipital scalp were utilized in all cases; in 13 individuals hairs were also obtained from the frontal and fronto-parietal scalp areas for comparison to occipital hairs.

Ninety-six patients,* whose ages ranged from 2–70 years, were included in this study. The majority (92%) had a diagnosis of disseminated cancer (including leukemia and lymphoma). The diagnoses of the remainder included basal cell carcinoma of the skin, psoriasis, rheumatoid arthritis, gout, and congenital myxedema. Hair samples were taken always from the occipital scalp region of these patients. Control examinations of hairs were done prior to the initiation of chemotherapy, and subsequent examinations were made at repeated intervals after therapy was begun. In 57 patients effects of the following drugs were studied: methotrexate, 3′,5′,dichloromethotrexate, triethylene thiophosphoramide (thio-tepa), 6-mercaptopurine, 5-fluorouracil, colcemid, actinomycin D, mechlorethamine (nitrogen mustard), cyclophosphamide (Cytoxan®), and Vinca leukoblastin, a plant alkaloid (5) presently undergoing preliminary clinical trials (6). All drugs were administered therapeutically.

Changes in hairs were compared to changes in peripheral white blood cell count, reticulocyte count, platelet count, and other signs of toxicity recorded by the physicians caring for the patients. In order to establish unequivocal evidence of drug effect on peripheral blood elements the degree of alteration of these elements was calculated, and only the following changes were accepted as significant.

1. A decrease in the number of circulating white blood cells to a value of less than 45% of the initial (pre-treatment) values, and to an absolute level of less than 4000 cells per cu. mm.

2. A decrease in the number of circulating platelets to less than 50% of the pre-treatment level.

3. A ten-fold or greater decrease in the percentage of circulating reticulocytes.

It is recognized that these criteria are perhaps somewhat rigid, but they were employed in order to facilitate reliable correlation of hair changes with definitive alterations of the formed elements of the blood.

RESULTS

Scalp hairs in normal individuals. The percentage of growing hairs in the occipital scalp of the 38 normal individuals was found to range from 66–99%, with an average of 86.5%, values which are in agreement with ones previously reported (4). In some individuals the percentage of growing hairs in other scalp regions was markedly different from the occipital region, the frontal and fronto-parietal areas generally having lower percentages. This difference was greatest in two men with advanced frontal balding, in whom the frontal scalp was found to have respectively 44% and 36% fewer growing hairs than the occipital region. Increased numbers of resting hairs also have been found in balding scalps by histologic means (7). Some non-balding

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females in this study, however, also had decreased numbers of growing hairs in the anterior scalp regions, though the differences were much less.

The percentage of hairs epilated with the root sheaths attached varied widely and did not correlate with age or sex of the individuals. The contour of the keratogenous zone was basically similar in all individuals.

Scalp hairs in patients not receiving chemotherapy. The percentage of growing hairs in the occipital scalp of persons with illnesses listed above, and who were not receiving a cancer chemotherapeutic drug at the time, was found to range from 35–100%, with an average of 85.5%. The range is greater than that found in normal scalps, and is in agreement with previously published figures (4). Except for the increased percentage of resting hairs in some of these patients no other abnormality in hairs was found, the structural appearance of the roots not differing significantly from the normal.

The percentage of growing hairs from a single examination was shown to be reliable when the results of repeated examinations on a group of 53 patients not receiving chemotherapy were analyzed. The average number of examinations per patient was 6 (range of 2–33). The examinations were repeated over periods of 1–30 weeks. In only three of the 53 persons did the percentage of growing hairs at any time deviate more than ±10% from the mean in each case. The average deviation in all patients was ±4.8%.

Changes in Hair Roots Following Chemotherapeutic Drugs

Methotrexate. Methotrexate, as all chemotherapeutic drugs, visibly affects growing (anagen) hairs only, and not hairs in the quiescent (telogen) stage of the growth cycle. The effect of usual therapeutic doses of methotrexate on the growing hairs of the scalp may be characterized by an inhibition of growth of the hair root, which is reversible when drug adminis-

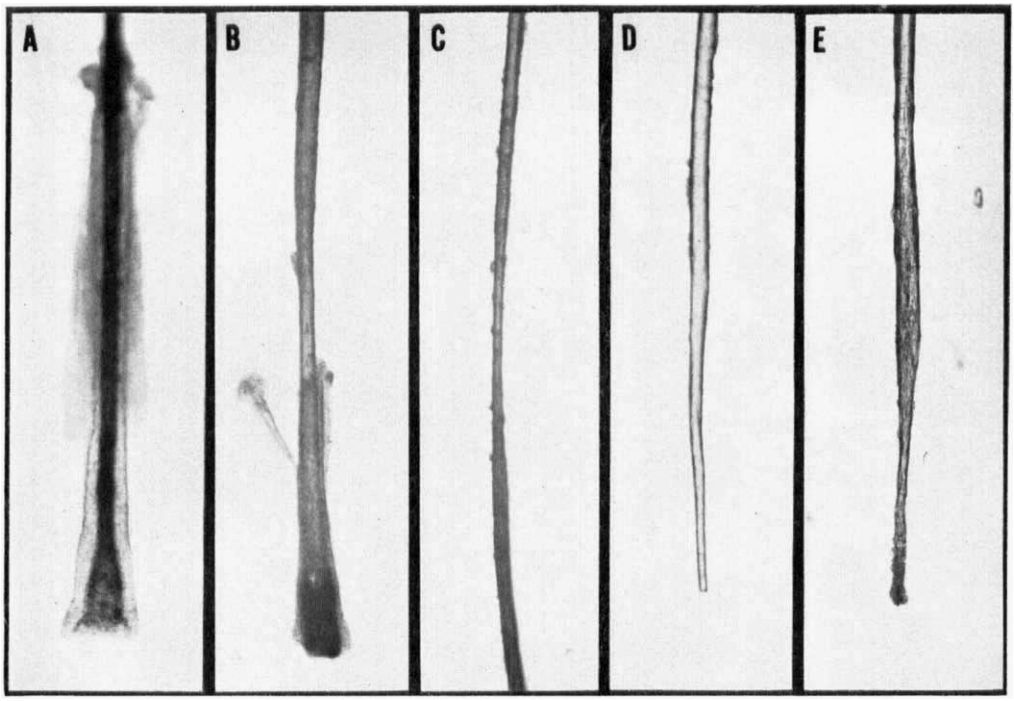


FIG. 2. Appearance of epilated scalp hairs which show the effect of chemotherapeutic drugs. A. Hair root four days following the administration of methotrexate. The hair bulb and keratogenous zone are diminished in diameter. B. Constriction of the hair shaft 7 days following methotrexate administration. Production of normal hair shaft proximal to constriction indicates that the germinative matrix (lowermost) has recovered from the drug effect. C. Constriction in distal hair shaft indicative of methotrexate therapy in the past. D. Hair shaft broken off at the point of severe constriction. E. Marked drug effect with atrophy of the growing root. This hair will fall out but will be replaced by a new hair.

TABLE I

Comparative effects of Methotrexate on scalp hair, oral mucosa, and bone marrow

Patient	Percentage of Growing Hairs Affected	Oral Ulceration	Platelet Decrease	Reticulocyte Decrease	WBC Decrease	Dose of Methotrexate
(1) R. R.	0	0	0	0	0	2.5 mg./day p.o. × 10
(2) J. S.	100	+	+	+	+	5.0 mg./day p.o. × 3
(3) I. L.	100	0	+	+	+	5.0 mg./day p.o. × 13
(4) R. R.	0	+	+	ND*	0	5.0 mg./day p.o. × 53
(5) O. G.	0	0	0	0	0	7.5 mg./day p.o. × 30
(6) O. R.	0	+	+	+	0	7.5 mg./day p.o. × 15
(7) O. R.	0	0	+	+	0	7.5 mg./day s.c. × 16
(8) J. H.	0	0	+	0	+	7.5 mg./day p.o. × 17
(9) I. A.	72	+	0	0	0	10 mg./day p.o. × 6
(10) D. S.	100	+	+	+	+	10 mg./day I.V. × 5
(11) M. M.	100	+	+	+	+	25 mg./day I.V. × 5
(12) M. K.	98	+	+	+	+	25 mg./day I.V. × 4
(13) M. A.	85	+	0	+	+	25 mg./day I.V. × 5
(14) M. A.	79	0	0	+	+	25 mg./day I.V. × 5
(15) I. H.	100	+	+	+	+	25 mg./day I.V. × 5
(16) I. H.	100	+	+	+	+	25 mg./day I.V. × 5
(17) I. H.	100	+	+	+	+	25 mg./day I.V. × 5
(18) S. P.	100	+	+	+	+	25 mg./day I.V. × 5
(19) B. W.	100	+	+	+	+	25 mg./day I.V. × 5
(20) D. B.	100	+	+	+	+	25 mg./day I.V. × 5
(21) S. B.	100	+	+	+	+	25 mg./day I.V. × 5
(22) J. E.	100	+	+	+	+	30 mg./day I.V. × 5
(23) D. K.	63	+	+	ND	+	450 mg. I.V. × 1
(24) I. S.	100	+	+	+	+	500 mg. I.V. × 1
(25) E. B.	97	+	0	0	0	700 mg. I.V. × 1
(26) E. B.	100	+	0	+	0	840 mg. I.V. × 1
(27) L. B.	91	+	0	+	0	1000 mg. I.V. × 1

* Not done.

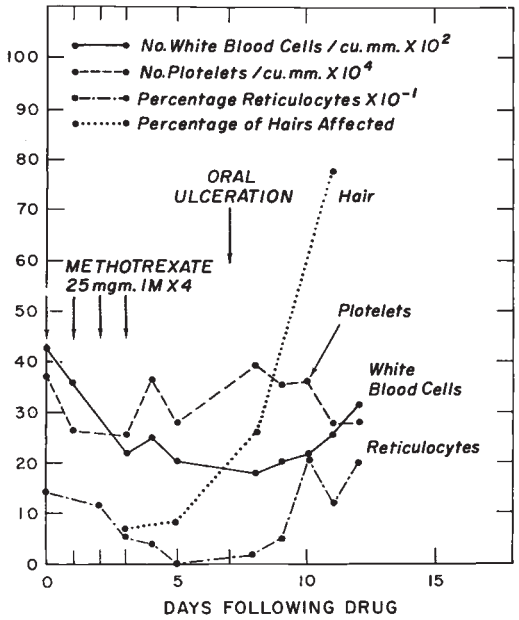
tration is discontinued (4). Four to six days following a *single* adequate dose of the drug the first change in the hair root recognized microscopically is a diminution of the diameter of the hair bulb and/or keratogenous zone (Fig. 2A). As the hair root recovers from the insult growth of the matrix resumes, and the shaft of the hair produced within the next 2–5 days is found to contain a segment with diminished diameter, *i.e.* a constriction of the shaft (Figs. 2B, 2C). (A twist in a hair that is oval in cross-section can be distinguished from a constriction by rotating the shaft with a fine forceps). The constriction moves distally as the hair continues to grow, moving progressively farther out from the hair root. Breakage may occur at the point of a pronounced constriction when the hair is pulled from the scalp, and such broken hairs are recognized microscopically by the tapering of the hair shaft at the point of breakage (Fig. 2D).

Several patients in this study received similar doses of methotrexate (25 mgm. intravenously on four successive days) on two or more occasions at approximately two-week intervals. Successive constrictions of the hair shaft, corresponding to the successive courses of drug, were demonstrated, the distance between the constrictions proportionate to the interval of time between courses of drug.

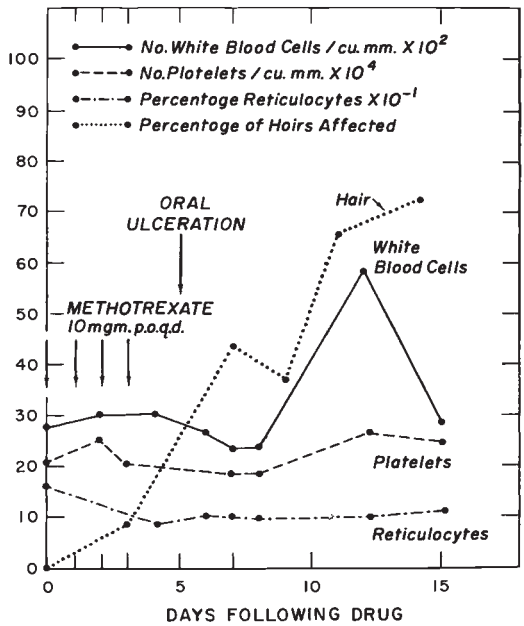
Mention should be made of a particularly marked effect of methotrexate on the hair roots of one patient (Table I, Case #2) in whom severe toxicity developed in association with impaired renal function. Total loss of scalp hair occurred, and the hair bulb portion of epilated hairs appeared to have approached complete atrophy (Fig. 2E). Regeneration of roots did occur, however, since regrowth of hair was clinically evident within four weeks of hair loss.

Serial examinations of epilated scalp hairs during and following 27 courses of methotrexate therapy were performed on 22 patients and the percentage of growing hairs showing the above signs of damage from the drug was determined in each instance. Evidence of drug effect on the bone marrow, determined by definitive decreases in the numbers of formed elements in the peripheral blood, was recorded. The presence of ulceration of the oral mucous membrane, a clinical sign of drug effect on normal tissue, *i.e.*, "toxicity" from the drug, was also noted.

Comparison of the data from these patients (Table I) reveals that damage of scalp hair roots is a sensitive indicator of toxic effects of methotrexate and may be as reliable as other more



GRAPH I. Correlation of hair damage with other signs of toxicity following methotrexate. (Patient M. A. #14, Table I.)



GRAPH II. Correlation of hair damage with signs of toxicity following methotrexate. (Patient I. A. #9, Table I.)

TABLE II

Comparative effects of drugs other than Methotrexate on scalp hair, oral mucosa and bone marrow

Patient	Agent	Percentage of Growing Hairs Affected	Oral Ulceration	Platelet Decrease	Reticulocyte Decrease	WBC Decrease	Dose
(1) S. S.	Dichloro-methotrexate	100	0	+	0	+	100 mg./day I.M. × 4
(1) C. R.	5-fluorouracil	0	0	0	0	0	3.4 mg./Kg./day I.V. × 14
(2) W. J.	5-fluorouracil	0	0	0	0	0	4 mg./Kg./day I.V. × 14
(3) E. D.	5-fluorouracil	99	0	+	+	+	6 mg./Kg./day I.V. × 8
(4) N. N.	5-fluorouracil	0	0	0	0	0	6 mg./Kg./day I.V. × 14
(5) J. J.	5-fluorouracil	0	0	0	0	0	6 mg./Kg./day I.V. × 14
(6) M. W.	5-fluorouracil	90	+	0	+	+	8 mg./Kg./day I.V. × 8
(7) I. S.	5-fluorouracil	100	+	+	+	0	15 mg./Kg./day I.V. × 5
(1) R. T.	Actinomycin D	85	0	0	0	0	20 μgm./Kg. I.V. × 1
(2) C. B.	Actinomycin D	0	+	+	0	0	50 μgm./Kg. I.V. × 1
(3) C. B.	Actinomycin D	0	0	+	0	0	50 μgm./Kg. I.V. × 1
(4) O. J.	Actinomycin D	0	0	0	ND*	0	50 μgm./Kg. I.V. × 1
(5) O. J.	Actinomycin D	0	0	+	ND	+	75 μgm./Kg. I.V. × 1
(6) D. S.	Actinomycin D	100	+	+	+	+	50 μgm./Kg. I.V. × 1
(7) B. D.	Actinomycin D	100	+	+	+	+	125 μgm./Kg. I.V. × 1
(1) M. H.	Thio-tepa	0	0	0	0	0	0.2 mg./Kg./day I.V. × 4
(2) V. C.	Thio-tepa	0	0	0	0	0	0.2 mg./Kg./day I.V. × 4
(3) V. A.	Thio-tepa	0	0	0	0	0	0.2 mg./Kg./day I.V. × 3
(4) W. G.	Thio-tepa	100	+	+	+	+	0.4 mg./Kg./day I.V. × 4
(1) I. F.	Colcemid	100	+	+	+	+	5 mg./day p.o. × 7
(1) S. K.	Cytoxan	0	0	0	0	0	15 mg./Kg. I.V. × 1
(2) S. K.	Cytoxan	100	0	0	+	0	20 mg./Kg. I.V. × 1
(1) M. A.	Vinca Leuko-blastin	100	+	+	+	+	12 mg./day I.V. × 3

* Not done.

commonly recognized signs. Each instance of hair damage was accompanied by at least one other sign of drug toxicity (see, for example, Graph I). In seven instances hair changes occurred in the absence of one or more of the usual parameters of toxicity. For example, two patients (#9, #25) with hair changes had no significant alteration in the levels of circulating white blood

cells, reticulocytes, or platelets (Graph II). Hair root changes were absent in four cases (#4, #6, #7, #8) having one or more signs of toxicity; it is interesting that in these patients levels of either reticulocytes or white blood cells were also unchanged.

Dichlormethotrexate. Hairs were examined from but one patient following therapy with this chlorinated derivative of methotrexate. Constrictions in the hair shafts indistinguishable from those due to methotrexate were found concommitantly with other signs of toxicity (Table II).

5-Fluorouracil. Seven patients receiving this drug in varying dosage were observed. Hairs from three of these showed signs of damage indistinguishable from those due to methotrexate. In each of these three individuals at least one of the common signs of toxicity was absent. In no case was there clinical or hematologic effect without recognizable hair changes.

Actinomycin D. Two of seven patients who received this drug developed signs of severe toxicity; in these two patients the matrix of all growing hair roots appeared completely atrophic. In another case, constrictions of the hair shaft like those following methotrexate were seen, although this patient showed no hematologic alterations. In three cases no hair changes were demonstrated despite other evidence of toxicity.

Triethylene thiophosphoramide. (Thio-tepa). One individual developed severe oral ulcerations and depression of hematologic elements. The roots of all growing scalp hairs appeared atrophied. Three other patients receiving smaller doses of the drug had no evidence of toxicity.

Colcemid. Alopecia occurred in one patient who received a total of 34 mgm. of colcemid orally over a period of seven days. Clinical and hematologic signs of toxicity were correlated with atrophy of the roots of all growing hairs. In one other patient receiving colcemid, in whom oral ulcerations and depression of circulating white blood cells developed, 22% of growing hairs were found to be broken at the keratogenous zone without evidence of constriction at this point.

Cyclophosphamide. (Cytoxan). One patient was observed who received two successive single intravenous doses of this drug, 15 and 20 mgm./kgm. of body weight respectively. With the smaller dose no clinical or hematologic signs of toxicity occurred, nor did any changes in hair roots. With the larger dose all growing hairs showed focal constrictions, yet of the hematologic elements only the reticulocyte count was diminished.

Vinca leukoblastin. Therapy with this plant alkaloid in one individual was followed by severe toxicity in all parameters. Correspondingly, all growing hair roots were atrophic. The atrophy of the roots resulting from this drug, as when it occurred in those instances following thio-tepa, methotrexate, colcemid, and actinomycin D, was followed by extensive alopecia; regrowth of hair within 4–6 weeks of hair loss was observed in all cases.

Hair changes following other drugs. Varying percentages (up to 22%) of hairs that were broken off at the keratogenous zone, similar to that seen in the one patient (above) following colcemid, were observed in several cases during combined chemotherapy (simultaneous or sequential administration of small doses of two or more chemotherapeutic agents). The same phenomenon occurred in some patients receiving nitrogen mustard or 6-mercaptopurine. However, no change of any kind appeared in hairs from four patients who received nitrogen mustard and in whom hematologic depression occurred All of these patients had either leukemia or Hodgkin's disease, and whether the hair changes, when present, were associated with the drugs, the specific disease, or unknown factors is not apparent.

In no patient receiving any of the drugs included in this study did the percentage of resting hairs increase over pre-treatment values.

DISCUSSION

The variation in the percentages of growing hairs from different areas of the scalp in an individual, compared to the relatively constant percentage of growing hairs in the occipital scalp, emphasizes the need for using hairs from one pre-selected areas in a study such as presented here.

Data derived from occipital hairs confirms the observation that increased numbers of resting hairs is associated with illness in some persons. It seems important to re-emphasize also that no increase in numbers of resting hairs was observed following therapy with any of the drugs. Since these drugs affect growing hairs only, however, hairs lost as a consequence of therapy are predominantly growing hairs; thus the *relative* percentage of resting hairs remaining in the scalp rises. Hence, in the extreme circumstance of severe drug toxicity where all growing

hairs are affected most scalp hair is lost. Because the hairs remaining in the scalp must all be resting, it is apparent that examination of the patient at this time may lead to the erroneous impression that the drug caused conversion of hairs from the growing to the resting stage.

Although closer study, particularly with serial histologic examinations of biopsy specimens of scalp, might be expected to reveal that certain anti-tumor drugs damage the hair root and interfere with the growth of hair via mechanisms that are characteristic for each drug or group of drugs, no such differences could be ascertained from microscopic examination of epilated hairs. The essential effect of each drug, whether alkylating agent, anti-metabolite, or mitotic inhibitor, seemed to be inhibition of growth of the hair root that was promptly reversible when the drug was discontinued. In those instances where the insult was great enough to cause marked atrophy of the hair bulb, precipitous loss of hair occurred. Regrowth of hair was clinically visible within 4–6 weeks.

While damage to hair following methotrexate was associated with larger amounts of the drug and with the appearance of clinical and hematologic signs of toxicity, there was no linear correlation of absolute dose administered with any sign of drug toxicity. However, the most pronounced hair root damage, marked atrophy, invariably was associated with most severe toxicity from methotrexate as measured by the other signs. This correlation was also found with other drugs.

Since the major route of excretion of methotrexate is renal (8), systemic accumulation of the drug may result from impaired renal function, and severe toxicity may occur following small doses in patients with renal failure (8, 9). This circumstance prevailed in patient J. S. (Table I, *2).

Clinically evident hair loss following drugs may occur by two different mechanisms. Hairs with atrophied roots are lost readily, either falling out spontaneously or removed by such casual procedures as combing the hair. Hairs with marked constrictions of the shaft, on the other hand, break off easily at the point of constriction. The root however remains in the scalp, since it has already recovered from the insult of drug and has produced the hair shaft containing the constriction. Therefore, the magnitude of damage to hair roots, and hence degree of toxicity, cannot be ascertained from the occurrence of hair loss alone but is best determined from the microscopic appearance of epilated hairs.

The presence of definite signs of toxicity in scalp hairs of patients who lack one or several of the more commonly used signs of toxicity suggests that the hair root is a rather sensitive indicator of toxic effects of many cancer chemotherapeutic drugs. Examination of epilated scalp hairs can be of value in ascertaining the presence or degree of toxicity in some patients receiving these drugs.

SUMMARY

Structural damage of human scalp hairs occurs following the administration of a variety of cancer chemotherapeutic drugs. A close correlation is found between hair root damage and hematologic signs of drug toxicity. The hair is a sensitive indicator of the toxic effects of these drugs. Examination of hair roots can be useful in the clinical management of patients receiving chemotherapeutic drugs.

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